U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 22.

B. T. GALLOWAY, Chief of Bureau.

# INJURIOUS EFFECTS

OF

# PREMATURE POLLINATION;

WITH

## GENERAL NOTES ON ARTIFICIAL POLLINATION AND THE SETTING OF FRUIT WITHOUT POLLINATION.

BY

CHARLES P. HARTLEY,

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VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS.

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[a] Detailed to Botanical Investigations and Experiments.
[b] Detailed to Seed and Plant Introduction.

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# LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
*Washington, D. C., May 22, 1902.*

Sir: I have the honor to transmit herewith a paper on the Injurious Effects of Premature Pollination, with General Notes on Artificial Pollination and the Setting of Fruit without Pollination, and respectfully recommend that it be published as No. 22 of the Bureau series of bulletins. The paper was prepared by Mr. Charles P. Hartley, assistant in physiology in the Plant-Breeding Laboratory, Vegetable Pathological and Physiological Investigations, and was submitted by the Pathologist and Physiologist.

Respectfully,

B. T. Galloway,
*Chief of Bureau.*

Hon. James Wilson,
*Secretary of Agriculture.*

# PREFACE.

The importance which plant breeding has assumed in the improvement of varieties of cultivated plants makes it desirable that careful attention be given to every detail involved in the production of hybrids. The accompanying paper, by Mr. Charles P. Hartley, assistant in physiology in the Plant-Breeding Laboratory, Vegetable Pathological and Physiological Investigations, is the result of a study of the effects of premature pollination in its relation to the setting of fruit. The investigation has demonstrated the decidedly injurious effect of premature pollination in several cases, and the fact is emphasized that in order to secure successful results in the production of hybrids and the setting of fruit, careful consideration must be given to the time when pollen should be applied to the stigma. It is believed that the results here presented will be of service to investigators and to workers in plant breeding.

ALBERT F. WOODS,
*Pathologist and Physiologist.*

OFFICE OF THE PATHOLOGIST AND PHYSIOLOGIST,
*Washington, D. C., May 21, 1902.*

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## WITH GENERAL NOTES ON ARTIFICIAL POLLINATION AND THE SETTING OF FRUIT WITHOUT POLLINATION.

### INTRODUCTION.

It is quite generally taken for granted that if pollen is placed on the stigmas of flowers some time before they are receptive it will simply remain there until the stigmas become mature, and then germinate and fertilize the flowers. Darwin,[a] in speaking of the various kinds of flowers with which he experimented in order to show the devitalizing effect of self-fertilization, says:

> As the flowers which were crossed were never castrated, it is probable or even almost certain that I sometimes failed to cross-fertilize them effectually, and that they were afterwards spontaneously self-fertilized. This would have been most likely to occur with dichogamous species, for without much care it is not easy to perceive whether their stigmas are ready to be fertilized when the anthers open. But in all cases, as the flowers were protected from wind, rain, and the access of insects, any pollen placed by me on the stigmatic surface while it was immature would generally have remained there until the stigma was mature, and the flowers would then have been crossed as was intended.

Such would perhaps have been the case with some flowers, but the experiments herein described show that it is not true of all. On the contrary, they furnish proof for a statement to the effect that a premature pollination is destructive to the flowers of some plants, preventing them from forming seeds. The experiments and microscopic examinations show that with tobacco at least premature pollination not only results in a failure of the flower to set seeds, but that the growth of the pollen tubes into ovaries before the ovules are mature enough for fertilization results in an injury which causes the flowers to immediately fall.

The facts that tobacco flowers fall in about thirty-six hours after pollen is applied to the immature pistils, whether the flowers be previously emasculated or not, and that they do not fall if emasculated and not pollinated, nor if pollinated with other pollen than tobacco

[a] Cross and Self-Fertilization in the Vegetable Kingdom, p. 23.

pollen, and further, that pollen tubes are found penetrating the styles and ovaries of the fallen flowers, give good reasons for the statement that the application of pollen to the pistils of some flowers before the pistils are mature enough to receive the pollen produces an injury which results in the death of the flowers.

In working with the different kinds of flowers herein mentioned, except *Datura tatula*, the following method was employed: Ten similar flower buds were selected and treated in exactly the same manner and labeled by means of numbers, the ten flowers of each experiment bearing the same number. When the results were to show the effects of the different methods of treatment, the experiments were performed at the same time and on similar flowers equally distributed on the same plants. This made all conditions alike for the experiments to be compared, except the treatment of the blossoms.

At the end of the remarks regarding each kind of flower, a table is given describing all the experiments with that flower. In these tables, as elsewhere, the experiments are designated by the original numbers attached to the flowers when the work was begun.

## EXPERIMENTS WITH TOBACCO BLOSSOMS.

The conclusions in regard to the work with tobacco blossoms are drawn from the behavior of 670 hand-pollinated flowers of Cuban tobacco (*Nicotiana tabacum*). The different questions or phases of the work were tested by experiments consisting in every instance of 10 similar flowers, making in all 67 experiments. Quite often several of these numbered experiments were exact repetitions and as such their results compare favorably. By combining these like experiments we get results based upon work with larger numbers of flowers. Thirty-two of the 67 experiments, while throwing light on the questions here considered, were made primarily for the purpose of settling other questions and to test the longevity of tobacco pollen, and so will not be referred to in this article. This will explain why the experiments with tobacco blossoms given in Table I are not numbered consecutively. It may be mentioned here that pollen that had been kept in tinfoil for three months, while still capable of producing germinative seeds, was not so destructive to immature pistils as was fresh pollen, perhaps because the old dry pollen germinated less readily on the young stigmas before the exudation of stigmatic fluid which indicates the receptive period. At one time it was thought that this explanation was corroborated by the fact that no seed pods resulted from pollen two months old when used on mature flowers in the field, while pollen three months old gave good results on similar flowers in the greenhouse. It was thought that perhaps the dry pollen germinated with greater ease in the moist greenhouse. Such may have been the case, but it is now thought that the hot summer weather affected the pollen. The pollen

used in the field, though only two months old, was exposed to, and perhaps injured by, the hot weather of July and August, while that used in the greenhouse experienced the cooler weather of April, May, and June. When fresh pollen was used the destructive effect of premature pollination was as pronounced on the flowers in open air as on those in the greenhouse. That a saturated atmosphere is sufficient to start the growth of tobacco pollen was shown by placing some on the under surface of a cover glass over a drop of water in the well of a microscopic slide. The next day pollen tubes were found growing from many of the grains.

The seeds resulting from these experiments were not all planted to test their germination, but seeds from several of the tobacco experiments were tested and the large plump ones (Pl. III, fig. 6), which, when crushed, showed that they contained oil, germinated well, while the smaller, light, chaffy ones failed to germinate. It was therefore thought unnecessary to test the germination of all the seed, and good ones were distinguished for the most part by their appearance.

When the first flowers were operated upon—i. e., those of experiments 1, 2, and 3—very little attention was paid to the age of the flowers chosen, other than to see that they had not yet opened. This is now known to account for the great variation in the number of pods obtained. (See Table I.) As the work proceeded, however, it was found that the age of the flower at the time of pollination was of the utmost importance in order to obtain results of value, and the time was recorded in hours in performing all experiments after the first six. Finding that the age of the flower operated upon was the most vital condition entering into the experiments, a very careful study of the growth of the flowers was made. A number of young flower buds of various ages were labeled with tags upon which were placed the date and size of the bud, and each day thereafter the size and condition of these buds and the resulting flowers and seed pods were written on the respective labels. This was repeated several times. The weather conditions were found to exert a marked effect on the rapidity of development, but it was noticed that the size and appearance of the corollas were good indications of the advancement of the inclosed anthers and stigmas, so that consequently the corollas were used in the experiments as a means of designating the age of the flower buds operated upon. The 35 experiments performed with tobacco to settle the destructive influence of premature pollination are outlined and their results given in Table I (p. 17). Tobacco being a self-fertilizing flower, the stigma is receptive at the time the anthers open, and the anthers usually begin to shed pollen when the flower begins to open.

Comparing the length of the corolla with the length of time before the corolla is fully open and the stigma receptive, we find the following conditions to exist when the plants are thrifty and the weather fairly favorable:

Four days before the stigma is receptive the corolla does not extend beyond the calyx lobes, which are one-half inch long. The flower labeled *c*, in Pl. I, fig. 1, is in this stage of development and is the same as those which were chosen for experiments 22 and 35. (See Table I.) The growth of an unmolested bud of this age can be studied by comparing the small unlabeled bud on the right in Pl. I, fig. 1, with resulting flower and seed pod in Pl. I, fig. 2, and Pl. II, fig. 1, respectively.

Three days before the stigma is receptive the corolla is one-fourth to one-half inch longer than the calyx (flowers *d* and 6, Pl. I, fig. 1). Flowers of this age were chosen for 10 of the experiments given in the table.

Two days before opening, the corolla is three-fourths to 1 inch longer than the calyx, as shown by flowers 1 and 2 in Pl. I, fig. 1. Flowers of this age, as well as younger ones, were found to be killed by pollination. (Compare figs. 1 and 2, Pl. I.)

One day before opening, the corolla is $1\frac{1}{4}$ to $1\frac{3}{4}$ inches longer than the calyx, the terminal one-fourth inch being of a red color. Flowers 3, 4, 5, and *a*, Pl. I, fig. 1, are of this age and can be pollinated with fair success (experiments 4, 6, etc.), but the results will be much better if the work is done twelve hours later (as in experiments 16 and 36), or if the flowers are emasculated at this age, or younger, and pollinated when the pistils become receptive (experiment 7 or 20).

The next day the corolla and anthers open at the same time and the receptive pistil is of proper length and maturity to receive the pollen directly from the opening anthers. One month after pollination the seed pod is ripe.

Having obtained this data in regard to the manner of growth, the flowers were designated in the notes made at the time the experiments were performed as "three days before pistils would have been receptive," "one day before pistils would have been receptive," etc.

By the time the results of the first 10 experiments were obtained it was definitely known that an abundance of fresh pollen applied to tobacco flowers two or three days before the pistils become receptive results in the falling of the flowers without setting fruit, while flowers operated upon one day before becoming fully open and receptive give fair percentages of fruits, as shown by experiments such as Nos. 7 or 8. But it was necessary to find out why the former failed while the latter gave fair percentages. It might be supposed that the younger flowers were more injured by emasculation, but several experiments soon proved that this is not the case. In experiment 20 the flowers were emasculated three days before becoming receptive and pollinated when receptive, resulting in 100 per cent of fruits, while in experiment 21 they were emasculated and pollinated three days before becoming receptive, resulting in no fruit whatever. The only difference in experiments 20 and 21 is in the time of pollination. Other

experiments, for example Nos. 18 and 19, which are described in Table I (p. 17), prove that the falling of the young flowers is not in any way caused by the early emasculation, while in experiments 35, 38, and 40 they fell soon after being prematurely pollinated, although they had not been emasculated.

A study of figs. 1 and 2, Pl. I, will also show that the falling of the flowers is not associated with the emasculation. For fig. 1 the photograph was taken immediately after emasculating and pollinating the flowers, and for fig. 2 the photograph was taken forty-eight hours later. The lettered flowers on the right-hand stem of the plant were simply emasculated at the same time the numbered flowers on the left-hand stem were emasculated and pollinated. The prematurely pollinated flowers, 1, 2, and 6, shown in these illustrations, had all fallen within forty or less hours of the time they were pollinated, while those of the same age, *a*, *c*, and *d*, that were emasculated and not pollinated, continued to grow. These flowers were found fallen at 9 o'clock in the morning, the exact time of their falling not being known; but three other tests made at different times showed that in good growing weather such prematurely pollinated blossoms fall in about thirty or thirty-six hours after being pollinated. Flowers 3, 4, and 5 were treated exactly as were flowers 1, 2, and 6, but set seed instead of falling, because they were almost mature when pollinated. Fig. 1, Pl. II, is from a photograph of the same plant as shown in Pl. I, taken eight days later than fig. 1, and shows the seed pods which grew from flowers 3, 4, and 5, while the lettered ones that were emasculated but not pollinated are just fallen, making a difference of six days between the falling of those prematurely pollinated and those not pollinated.

There was always a great difference noticeable in the manner of falling of the prematurely pollinated blossoms and those that fell from other causes, such as non-pollination. When the fall was the effect of premature pollination, the separation of the flower from the plant was rapid and complete and not accompanied by any previous wilting of the flower, but invariably occurred at a joint situated at the base of the peduncle shown at *a*, Pl. III, fig. 3. Flowers 1 and 2, Pl. III, fell within some time less than thirty-nine hours after being pollinated, while flowers 3 and 4, which were emasculated at 12 m., February 27, and not pollinated, had not yet fallen at 9 a. m., March 5, when they were cut and photographed.

While comparing blossoms that had been emasculated and prematurely pollinated with similar ones that had been emasculated and bagged but not pollinated, the persistence with which the pistils retained their fresh receptive appearance was very noticeable and led to a few slight tests of the length of time the pistils could remain receptive and still result in the production of seed when pollinated. By referring to experiment 18 in Table I, it will be noticed that the 10 flowers emasculated June 7 and found receptive June 9 were not

pollinated till June 14, at which time the corrollas of the flowers were dead or badly withered, while the pistils were still fresh and receptive. On June 20 five pods were found growing, but they did not appear as thrifty as those of experiment 16 in which the pistils were pollinated at the proper time. In experiment 9 the pistils became receptive May 13, but were not pollinated until the next day, when pollen that had been left in the laboratory for three days was applied. The stigmas at the time of pollination were covered with a large drop of stigmatic fluid, in which the pollen seemed to dissolve and disappear from view. The experiment resulted in the setting of but three pods. It is thought that the reason for so poor a percentage of seed pods is the placing of the dry pollen in so large a drop of stigmatic fluid, perhaps causing the pollen grains to swell and burst; but this is only conjecture, for no attempt was made to ascertain the true behavior of the pollen grains.

In experiment 37 the pollen was placed on about one-fourth the entire surface of receptive stigmas and on only one side of the small suture tnat divides the stigma into equal parts. While the pods were still green four or five showed a marked one-sidedness, but when they had ripened and were cut for examination they all seemed as symmetrical as the general crop of pods. On cutting them, however, the good plump seeds were almost altogether in one-half of the ovary, the other half containing light, chaffy, undeveloped seeds.

It being fully determined that pollen applied to the entire stigmatic surface of premature pistils results in the falling of the flowers, some tests were made to determine the effects of smaller quantities of pollen. In experiment 41 fresh pollen was applied to one-fourth of the stigmatic surfaces of pistils that would have been receptive in three days, and the flowers were bagged without emasculation. Four days later but one flower had fallen, while it is safe to say that had the stigmas been entirely covered with pollen 90 per cent would have fallen. They were naturally self-pollinated and 8 set pods. No explanation is known why an occasional prematurely pollinated flower failed to fall (experiments 39 and 40) unless it be that accidentally a portion of the stigmatic surface escaped pollination and the flowers continued to grow because there was still a chance for them to set some seeds. Experiments 44 and 46 (see table) were treated as was experiment 41, except that the flowers were emasculated. But two pods set in experiment 44 and four in experiment 46; the other flowers withered and fell, not because a small quantity of pollen was applied prematurely, but because of a lack of pollination after becoming receptive. In experiment 37 the pollen was placed on only one side of the medial suture and seeds were found mostly in one-half of the pods, while in experiments 44 and 46 it was placed across the stigma in a line at right angles to the suture, and the well-developed seeds were found scattered among the undeveloped ones in both halves of the seed pod. From the partition which divides the ovary (Pl. III, fig. 7) a sharp line can be traced up

both sides of the pistil and be seen to terminate in the suture just mentioned. That there is a close relation between the pollination of one-half of the stigma and the setting of seeds in the corresponding half of the ovary is certain, but no attempt was made to ascertain whether pollen grains placed on one-half of the stigma are ever able to fertilize ovules in the opposite half of the ovary. The fact that pollen tubes all descend in the delicate conductive tissue occupying the center of the pistil might indicate that the tubes are free to grow to any portion of the ovary, but stained sections show that they grow parallel with one another and seem to follow the long, thin-walled cells of the conductive tissue (Pl. II, fig. 3). In the ovary the tubes make many short turns in various directions (Pl. II, fig. 5).

While flowers two days before opening fall as completely as any, when their stigmas are entirely covered with pollen, they nevertheless recover from a slight premature pollination better than younger flowers. This can be noticed by contrasting experiments 44 and 46 with experiment 47.

As will be noticed in the tables, several substances were substituted for pollen. This was done in an effort to find if other substances could injure the stigmas and cause the flowers to fall as in the case of premature pollination. Nothing besides pollen was found to have this effect. Its damage goes deeper than the stigma.

As a mere irritation of the stigma is thought to have a tendency to cause some flowers to set fruit, it may not be amiss to view these experiments with this in mind. Of 60 emasculated flowers that had their stigmas covered with substances other than pollen, 14 set fruits, while of the 20 that were emasculated but never pollinated 2 set fruits. As a general thing the capsules resulting from the flowers that were not pollinated, and likewise those resulting from flowers whose stigmas were covered with some substance other than pollen, contained only small compressed undeveloped seeds, but the two pods obtained in experiment 52 by treating the fully receptive stigmas with magnesium sulphate contained some spherical seeds of almost full size which looked like good seeds, but when cut into proved to be hollow spheres.

By combining like experiments in which flowers of various ages were plentifully pollinated with fresh pollen, we get the following percentages of seed pods, of which it can be said that none resulting from the pollination of flowers earlier than one day before opening contained germinative seeds:

| | Per cent. |
|---|---|
| From 20 flowers pollinated four days before opening, 1 seed pod set. | 5 |
| From 40 flowers pollinated three days before opening, 2 seed pods set. | 5 |
| From 20 flowers pollinated two days before opening, 0 seed pods set. | 0 |
| From 40 flowers pollinated one day before opening, 31 seed pods set. | 77 |
| From 20 flowers pollinated one-half day before opening, 19 seed pods set | 95 |
| From 20 flowers pollinated when fully receptive, 19 seed pods set | 95 |

Dividing these into two groups, namely, those pollinated more than one day before opening and those pollinated one day or less than one day before opening, they show that the former set 4 per cent of seed pods which contained no germinative seeds, while the latter set 86 per cent of seed pods which contained germinative seeds.

## MICROSCOPIC STUDY OF TOBACCO PISTILS AND OVARIES.

Soon after becoming detached from the plant some of the prematurely pollinated blossoms were carried to the laboratory and the stigmas and pistils examined. That the pollen grains had germinated and penetrated the stigma could be seen, but the growth of the pollen tubes down the style could not be followed in the fresh material. This led to the premature pollinating of one series of blossoms and the mature pollinating of another series for microscopic study. The flowers of both these series were emasculated and kept bagged so that no pollen reached their stigmas except that purposely placed there. By this means the rapidity of growth of the pollen tubes was to some extent determined. At various intervals some flowers of each series, as well as some of various ages that had never received pollen, were cut and killed in Flemming's chromic-aceto-osmic acid solution and carried through alcohols and imbedded, sectioned, mounted, and stained with the safranin-gentian-violet triple stain. By this means the pollen tubes could be found penetrating the stigmas (Pl. II, fig. 2), in the styles (fig. 3), entering the ovary and dispersing among the ovules of the prematurely pollinated flowers (figs. 4 and 5).

Flowers collected twenty-two hours after being prematurely pollinated showed pollen tubes on and penetrating the stigma and some a short distance into the style but none in the ovaries, while in ovaries of flowers that had fallen because of being prematurely pollinated pollen tubes were abundant. Figs. 4 and 5 show pollen tubes in the ovaries of such flowers. In the sections that have been photographed pollen tubes are more abundant than they appear in the photomicrographs, but do not show because they are not in the same focusing plane.

Although the ovaries of the prematurely pollinated flowers contain many pollen tubes, none has been found entering the micropyle of an ovule, and in just what way the flower is destroyed has not been determined; but the kindness of Dr. Webber in examining the sections enables the writer to state definitely that the ovules, though encompassed with pollen tubes, are much too immature for fertilization, the embryo sacks being in the one and the two celled stages. The study of an abnormal condition of this kind may help to explain the attractive force which enables pollen tubes to find and enter ovules at the proper time.

Table I.—*Experiments with tobacco blossoms.*

[Each experiment performed upon 10 similar flower buds.]

| Experiment No. | Pistil would have been receptive in— | When emasculated. | When pollinated. | Remarks regarding pollen and pollination. | When examined, with remarks on condition. | Number of fruits. | Remarks. |
|---|---|---|---|---|---|---|---|
| | *Days.* | 1900. | 1900. | | | | |
| 1 | 1 to 3 | Apr. 26 | Apr. 26 | Fresh pollen applied abundantly | May 10 and May 21; pods were ripe May 21. | 6 | Flowers varied in age. |
| 2 | 1 to 3 | do | do | do | May 10; pod was ripe May 21 | 1 | Quite young flowers used. |
| 3 | 1 to 3 | Apr. 30 | Apr. 30 | do | May 10; May 21, large and green; June 8, ripe. | 8 | Flowers varied in age. |
| 4 | 1 | May 11 | May 11 | do | May 21; June 8, 7 ripe pods | 7 | |
| 5 | 1 | do | do | Only a few grains of fresh pollen applied to each pistil. | May 21, ovaries black but not fallen loose. | 2 | Only 9 flowers pollinated. |
| 6 | 1 | do | do | Fresh pollen applied abundantly | May 21; June 8, pods were ripe | 7 | |
| 7 | 1 | 3 p. m., May 11 | 11 a. m., May 12 | Pollen 1 day old applied abundantly | do | 9 | When pollinated 2 pistils showed injury. |
| 8 | 1 | 10 a. m., May 12 | 10 a. m., May 12 | do | do | 8 | |
| 9 | 1 | 11 a. m., May 12 | 11 a. m., May 14 | Pollen 3 days old applied abundantly | May 21, pistils dead but not fallen | 3 | Large drop of stigmatic fluid on stigma when pollinated. |
| 10 | 2 | 4 p. m., May 12 | 4 p. m., May 12 | Pollen 1 day old applied abundantly | May 21, all found fallen | 0 | |
| 11 | 2 | 10 a. m., May 12 | 10 a. m., May 22 | Fresh pollen applied abundantly | June 4, all found fallen | 0 | |
| 16 | ½ | 2 p. m., June 4 | 2 p. m., June 4 | do | June 14, 10 good growing pods | 10 | |
| 18 | 2 | 11 a. m., June 7 | 12 m., June 14 | do | June 9, full size and receptive | 5 | Pods lack vigor. |
| 19 | 3 | 10.30 a. m., June 7 | 2 p. m., June 9 | do | June 20, 9 thrifty seed pods | 9 | Tenth one died before pollinated. |
| 20 | 3 | 3 p. m., June 9 | 11 a. m., June 12 | do | June 20, 10 thrifty growing pods | 10 | Were receptive when pollinated. |
| 21 | 3 | 4 p. m., June 9 | 4 p. m., June 9 | do | June 12, 11 a. m., all 10 found fallen | 0 | Fallen flowers fixed for study. (See Pl. II, figs. 4 and 5.) |
| 22 | 4 | 11 a. m., June 28 | 11 a. m., June 28 | do | July 11, only 1 grew after pollination | 1 | Very small pod, no seeds. |
| 35 | 4 | Never | 10 a. m., Oct. 30 | do | Nov. 1, 10 a. m., 4 had fallen | 0 | Grew but little after pollination. |
| 36 | ½ | 12 m., Oct. 30 | 12 m., Oct. 30 | do | Nov. 14, 9 good healthy pods, ripe Dec. 24. | 9 | Many good seeds. (See Pl. III, figs. 6 and 7.) |
| 37 | ½ | 2 p. m., Oct. 30 | 2 p. m., Oct. 30 | Fresh pollen applied scantily to one-half of stigmas. | Nov. 14, 10 small pods, 4 were one-sided. | 10 | A few good seeds. |
| 38 | 3 | Never | 11 a. m., Oct. 30 | Fresh pollen applied abundantly | Nov. 1, 10 a. m., all 10 found fallen | 0 | Compare with experiment 35. |

TABLE I.—*Experiments with tobacco blossoms*—Continued.

| Experiment No. | Pistil would have been receptive in— | When emasculated. | When pollinated. | Remarks regarding pollen and pollination. | When examined, with remarks on condition. | Number of fruits. | Remarks. |
|---|---|---|---|---|---|---|---|
| | *Days.* | 1901. | 1901. | | | | |
| 39 | 3 | 11 a. m., Jan. 5 | 11 a. m., Jan. 5 | Fresh pollen applied abundantly | Jan. 10, 9 fallen; Jan. 29, 1 pod still growing. | 1 | 9 grew but little after pollination. |
| 40 | 3 | Never | 11.30 a. m., Jan. 5 | ....do | Jan. 10, 8 found fallen; Jan. 29, 1 pod growing. | 1 | Pod small. |
| 41 | 3 | ....do | 11 a. m., Jan. 10 | Fresh pollen applied to one-fourth surface of stigmas. | Jan. 14, 1 found fallen; Jan. 29, 8 growing. | 8 | Self-pollinated Jan. 13. |
| [a] 44 | 3 | 2 p. m., Jan. 19 | 2 p. m., Jan. 19 | Fresh pollen applied to one-fourth surface of stigmas. | Jan. 29, 8 found fallen; Feb. 9, 2 growing pods. | 2 | Pods contained a few good seeds. |
| 45 | 2 | 11.30 a. m., Jan. 29 | 11.30 a. m., Jan. 29 | Corn flour substituted for pollen | Feb. 4, 9 a. m., none fallen, still receptive. | 3 | Pods small, seeds poor. (See Pl. III, figs. 9 and 10.) |
| 46 | 3 | 10 a. m., Jan. 31 | 10 a. m., Jan. 31 | Fresh pollen applied to one-fourth surface of stigmas. | Feb. 9, 5 found fallen; Mar. 13, 4 ripe pods. | 4 | Pods contained a few good seeds. |
| 47 | 2 | 12 m., Jan. 31 | 12 m., Jan. 31 | ....do | Feb. 9, 8 still growing; Mar. 8, 8 ripe pods. | 8 | Do. |
| 48 | 2 | 11 a. m., Feb. 4 | 11 a. m., Feb. 4 | Air-slacked lime substituted for pollen | Feb. 9, still fresh; Feb. 28, 6 found fallen. | 4 | 1 pod photographed. (See Pl. III, fig. 8.) |
| 49 | 2 | 10.30 a. m., Feb. 5 | 10.30 a. m., Feb. 5 | Azalea pollen substituted for tobacco pollen | Feb. 9, 8 still fresh and receptive; Apr. 8, 1 ripe. | 1 | Pod small and seeds chaffy. |
| 50 | 2 | 9 a. m., Feb. 15 | 9 a. m., Feb. 15 | Corn flour substituted for pollen | Feb. 28, 4 found fallen; Apr. 8, 4 ripe pods. | 4 | Pods half size, seeds chaffy. |
| 51 | 3 | Mar. 12 | Never | Emasculated and bagged, but never pollinated. | Apr. 8, 2 small pods, others withered | 2 | Pods one-half and one-third size, seeds chaffy. |
| 52 | ½ | ....do | Mar. 12 | Magnesium sulphate substituted for pollen | Apr. 8, 2 ripe pods (half size), others withered. | 2 | Seeds spherical, but hollow. |
| 53 | ½ | 1.30 p. m., Apr. 8 | Never | Emasculated and bagged, but never pollinated | Apr. 25, all found dead | 0 | Ovaries did not enlarge. |
| 54 | 1 | 9 a. m., Apr. 25 | 9 a. m., Apr. 26 | Magnesium sulphate substituted for pollen | May 24, all found dead | 0 | Do. |

[a] Experiments 42 and 43 were not recorded until the day after the work was performed; so results are not considered reliable.

## EXPERIMENTS WITH BLOSSOMS OF DATURA TATULA.

The destructive effect of a premature pollination having been found to be so pronounced in the case of tobacco flowers suggested a trial of its effect on a related plant. Some young jimson weeds (*Datura tatula*) were placed in pots and kept in a greenhouse, where they soon came into flower, but unfortunately did not bloom profusely enough to furnish similar flowers in sufficient quantity to admit of experiments such as were performed with tobacco flowers. The following method was therefore adopted: From day to day these plants were examined and flowers in various stages of advancement were operated upon and the records made on labels attached to the plants at the petiole of each flower. As the seed pods resulting from the labeled flowers ripened or dried up they were cut and a record made of the size of the capsule, the number and condition of the seeds, etc. On the completion of the work an examination and classification of these labels showed results similar to those obtained in the case of tobacco flowers, with the one exception that the capsules of the prematurely pollinated flowers did not fall loose from the plants, but hung on for several weeks, although the ovaries did not grow any after pollination and contained no seeds. Pods resulting from flowers to whose stigmas pollen had been applied at the proper time were of normal size—1½ inches in diameter—and full of good, plump seeds; while pods from prematurely pollinated flowers were scarcely larger than they were when the flowers were pollinated, usually one-eighth to one-fourth of an inch in diameter, and contained no seeds. The only instances in which good seeds were found in pods from prematurely pollinated flowers were those in which the stigmas had been pollinated when exceedingly young and the flowers not emasculated. In some cases of this kind, and the same is true of tobacco flowers, the very young stigmas overcame the results of the early pollination and the flowers were later self-pollinated and produced seed. Indeed, the work with Datura blossoms indicates fully as strongly as does that with tobacco flowers that there may be a stage a certain time before maturity when the flowers suffer more from premature pollination than they would suffer from an earlier pollination. It may be necessary for the pistils or stigmas to reach a certain stage of development before pollen tubes can penetrate them.

## EXPERIMENTS WITH COTTON BLOSSOMS.

While hand pollinations were being made in order to obtain hybrids between Sea Island and upland cottons, the question arose as to whether the pollen could be applied at the time of emasculation with as good results as if applied to perfectly mature pistils. If such could be done much labor would be saved. Accordingly the work was arranged

in experiments of 10 flowers each in order to compare the results of the two methods. The pollinating was done on Sea Island cotton with pollen of the Mit Afifi variety. With the exception of experiments 5 and 6 the cotton experiments were with flower buds that would have opened naturally the day after they were emasculated. Such a bud is shown by Pl. IV, fig. 13. Figs. 14 and 15 show how emasculation was performed. Experiments 5 and 6 were with very young buds that would have opened in four or five days. The flowers of experiment 5 were pollinated when emasculated and those of experiment 6 two days later. No fruits set in either experiment. The flowers of experiment 1 were pollinated when emasculated, one day before the flowers would naturally have opened, and produced but one small boll; while the flowers of experiment 2, as near like the others as could be chosen on the same plants and emasculated at the same time, were not pollinated until the next day, and produced seven good bolls. In order to obtain Mit Afifi pollen that was pure and that had not been mixed with other kinds of pollen by the visits of insects, the flowers were collected before opening, and in each case just one day before the pollen was applied to the flowers. Experiment 3 was an exact repetition of experiment 1 and produced no bolls; while experiment 4, an exact repetition of experiment 2, produced ten good bolls. These results are decidedly in favor of waiting until the pistils are fully mature before applying the pollen, but work similar to the above, performed in 1901 on two different varieties of upland cotton, has given results so different from those obtained with Sea Island cotton that further study of the cotton flower is necessary before its reaction to premature pollination is understood. Although differing from the results obtained with Sea Island, the work with upland varieties still speaks in favor of waiting until the pistils are mature before applying the pollen, not so much, however, in order to obtain a greater percentage of bolls as to obtain bolls of larger size and more symmetrical shape, many bolls from the prematurely pollinated blossoms being small and one-sided.

The work with the upland varieties was conducted in the same manner as that with the Sea Island, pollen of Sea Island being used in experiments 7, 8, and 9. The flowers of experiment 7 on the Braddy variety were pollinated when receptive and produced 10 good bolls. Those of experiment 8 were pollinated when emasculated and again the next day, and produced 5 bolls, 3 of which were larger on one side than on the other and hardly as large as the bolls of experiment 7. The flowers of experiment 9 were pollinated when emasculated and produced 9 small bolls, 4 of which were decidedly larger on one side than on the other.

Experiments 10, 11, and 12 were upon the King variety of upland cotton, and pollen of the same variety was used. The flowers of

experiment 10 were pollinated when receptive and produced 6 good bolls. Those of experiment 11 were pollinated when emasculated and again the next day and produced 10 small bolls, 5 of which were decidedly one-sided. The flowers of experiment 12 were pollinated when emasculated and produced 7 rather good bolls.

In making the second pollination of experiment 8 it was noticed that the pistils of the flowers in experiment 7 which had not been prematurely pollinated were longer than those of experiment 8, but they were not measured because it was then too late to determine whether they had been precisely of the same length when emasculated. To determine whether premature pollination checks the growth of young pistils, the pistils of experiments 10 and 11 were accurately measured at the time of emasculation, 8 of each experiment being each five-eighths of an inch and 2 three-fourths of an inch in length, so that the aggregate length of the pistils was $6\frac{1}{2}$ inches. The flowers of experiment 11 were pollinated twenty-one hours before they were fully open and again twenty-one hours later, when the pistils were again carefully measured and found to give an aggregate length of $8\frac{1}{8}$ inches, an increase of $1\frac{5}{8}$ inches since emasculation, during which time the pistils of experiment 10, which were not prematurely pollinated, had made an aggregate increase of $2\frac{1}{4}$ inches. This is to say that the application of pollen to 10 immature pistils reduced their growth during twenty-one hours by five-eighths of an inch.

The conclusion to be drawn from the cotton experiments is in favor of making an extra visit to the emasculated flowers to apply the pollen rather than to apply it when the flowers are emasculated. This is not only because larger percentages of fruits will result, but more especially because better fruits are obtained.

It is the opinion of the writer that the variation in the results obtained with Sea Island and upland cotton flowers similarily treated is largely due to the fact that the pistils were closely approaching a receptive condition. Of course this would cause no variation in the results, providing the size of the flowers and length of time before opening indicate the same stage in the development of the ovules of both kinds of cotton, but this may not be the case. The one-sidedness of many of the bolls resulting from early pollination indicates that only a portion of the ovules were mature enough for fertilization. Perhaps pollination a few hours earlier would have resulted in the falling of all the flowers. Tobacco flowers pollinated one day before opening give fair results, while those pollinated two days before opening are killed. It is thought that the same will be found true of cotton flowers, as indicated by the results of experiments 5 and 6, but more work with cotton blossoms is necessary before their behavior will be well understood.

TABLE II.—*Experiments with cotton blossoms.*

[Each experiment performed upon 10 similar flower buds.]

| Experiment number. | Pistils would have been receptive in— | When emasculated. | When pollinated. | Variety of cotton and kind of pollen used. | When examined, with remarks on condition. | Number of mature fruits. | Remarks. |
|---|---|---|---|---|---|---|---|
| | | 1900. | | | | | |
| 1 | 18 hours | 4 p. m., Sept. 12 | 4 p. m., Sept. 12 | Sea Island, Mit Afifi pollen 1 day old | Oct. 11, all fallen except 1 small boll. | 1 | |
| 2 | do | do | 11 a. m., Sept. 13 | do | Oct. 11, 7 bolls growing, 2 started but afterwards fell. | 7 | |
| 3 | 24 hours | 11.30 a. m., Sept. 13 | 11.30 a. m., Sept. 13 | do | Oct. 11, 8 set but soon died and fell. | 0 | |
| 4 | 20 hours | 2 p. m., Sept. 13 | 10 a. m., Sept. 14 | do | Oct. 11, 10 good bolls still growing | 10 | |
| 5 | 4 or 5 days. | 3 p. m., Sept. 13 | 3 p. m., Sept. 13 | Sea Island, fresh Mit Afifi pollen | Oct. 11, pistils did not grow to mature length. | 0 | |
| 6 | do | 3.30 p. m., Sept. 13 | 10 a. m., Sept. 15 | do | Oct. 11, pistils did not grow to mature length. | 0 | Pistils of fallen flowers were longer than in experiment 5. |
| | | 1901. | | | | | |
| 7 | 24 hours | 10 a. m., Aug. 28 | 10 a. m., Aug. 29 | Braddy, upland cotton, Sea Island pollen, 1 day old. | Oct. 9, 10 healthy bolls | 10 | Bolls average 2 inches long. |
| 8 | do | 11 a. m., Aug. 28 | 11 a. m., Aug. 28; 10.30 a. m., Aug. 29. | Braddy, upland cotton, Sea Island pollen, fresh for first pollination, and 1 day old for second. | Oct. 9, 5 fell soon after pollination. | 5 | Bolls average 1¾ inches long. |
| 9 | 22 hours | 12 m., Aug. 28 | 12 m., Aug. 28 | Upland cotton, fresh Sea Island pollen | Oct. 9, 1 fell soon after pollination. | 9 | Bolls average 1⅔ inches long. |
| 10 | do | 12 m., Aug. 29 | 10 a. m., Aug. 30 | King upland cotton, King pollen, 1 day old | Oct. 9, 4 fell soon after pollination. | 6 | Bolls average 1⅝ inches long. |
| 11 | 21 hours | 1 p. m., Aug. 29 | 1 p. m., Aug. 29; 10.30 a. m., Aug. 30. | King upland cotton, King pollen, fresh for first application, and 1 day old for second. | Oct. 9, 10 small, mostly one-sided bolls. | 10 | Bolls average 1⅖ inches long. |
| 12 | 18 hours | 3.30 p. m., Aug. 29 | 3.30 p. m., Aug. 29 | King upland cotton, King pollen, fresh | Oct. 9, 3 fell soon after pollination. | 7 | Bolls average 1¾ inches long. |

## EXPERIMENTS WITH ORANGE BLOSSOMS.

Only 15 experiments of 10 flowers each were performed with orange blossoms and the results were rendered somewhat unsatisfactory for two reasons: (1) The trees blossomed profusely, but set only a small quantity of fruit. Although no actual calculation was made, it was evident when the trees were examined, September 27, that had the total number of blossoms put forth in the spring produced as good a percentage of fruits as those experimented with, the trees would have yielded a much heavier crop. (2) Some portions of the trees produced a greater percentage of fruits than other portions. This was especially true of the older limbs, while the younger wood in the tops of the trees produced almost no fruit. Luckily, the records made when the experiments were begun designated the portions of the trees which the blossoms of each experiment occupied, thus explaining some apparent contradictions in the results. For example, experiment 9 (see Table III) should have given results similar to those of experiment 8, but unfortunately the blossoms of experiment 9 were located in a nonproductive portion of the tree and on the same limb as those of experiment 6, which resulted in no fruits, although it should have produced results similar to those of experiment 7 had its blossoms been located in as productive a portion of the tree.

This work was undertaken for the purpose of determining the effect of pollinating orange blossoms before the pistils became receptive. But an examination of blossom buds of different ages showed that the stigmas were receptive (if the presence of stigmatic fluid is a sure indication of receptiveness) in buds that would not have been fully open for nine days. Such a bud is shown by Pl. IV, fig. 4, and an emasculated bud of the same age is shown by fig. 5. The stigmatic fluid can not be easily distinguished in the photograph, but when the emasculated bud is held in the hand a drop of light-colored fluid is seen in the center of the stigma. The fluid is viscid and pollen adheres readily and abundantly to the very young stigmas. While these young buds did not possess the conditions of nonreceptiveness, they were so young that it was thought better to test the effect of pollinating them at this age rather than work with younger buds. The detailed results of the experiments can be obtained from Table III, which follows. The first nine experiments were performed with flower buds nine days before they would have been open, like the one represented in Pl. IV, fig. 4, and the other six with flowers four days older, like the one shown in fig. 3. The records suggest that better success results from work with the buds last mentioned, but it was demonstrated that fruits containing good, well-developed seeds will result from flowers pollinated nine days before they would naturally have received pollen. This was demonstrated with flowers of both seedy and seedless varieties. That the pollen so prematurely applied fecundated the ovules is proved by

the fact that the resulting fruits of trees of seedless varieties contained good seed which germinated almost as well as seeds of fruits from the same tree the flowers of which were pollinated at the normal time. Indeed, the seeds resulting from these premature pollinations germinated to a greater per cent than seeds taken at random from fruits of a seedy orange.

One might expect the seedlings resulting from the pollination of such immature pistils to show feebleness, but such is not the case. They are now 6 inches tall and growing with as much vigor as any in the seed bed. They also show polyembryonic tendencies to as great a degree as the other seedlings, having from one to as many as four separate plants growing from a single seed.

Seven fruits resulting from prematurely pollinated flowers of a Melitensis navel were compared with six from the same tree which resulted from flowers emasculated nine days before opening and hand pollinated when the pistils were fully mature. The following are the averages:

| Number of fruits. | Weight. | Diameter. | Number of seeds. | Seeds that germinated. |
|---|---|---|---|---|
| | *Grams.* | *Inches.* | | *Per cent.* |
| 7 | 183 | 2.91 | 5 | 73 |
| 6 | 242 | 3.10 | 8 | 87 |

The last four experiments were for the purpose of ascertaining whether seedy oranges are disposed to set fruit without pollination, as is customary with navel oranges. The 20 buds that were emasculated and bagged without pollination, as well as 20 that were emasculated and had their stigmas irritated by the application of magnesium sulphate, alike failed to set any fruits.

TABLE III.—*Experiments with orange blossoms.*

[Each experiment performed upon 10 similar flower buds.]

| Experiment No. | Flowers would have been fully open in— | When emasculated. | When pollinated. | Number pollinated, others having fallen before pollination. | Remarks regarding pollen and pollination. | When examined, with remarks on condition. | Number of ripe fruits. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| | *Days.* | | | | | | | |
| 1 | 9 | 10 a. m., Feb. 25 | 10 a. m., Feb. 25 | 10 | St. Michael, a seedy orange, pollen from same tree. | May 21, 5 fruits were growing | 2 | Seeds germinated well. |
| 2 | 9 | 11.30 a. m., Feb. 25. | 11.30 a. m., Feb. 25. | 10 | .....do | May 24, 4 fruits were growing | 2 | Do. |
| 3 | 9 | 2 p. m., Feb. 25 | 3 p. m., Mar. 6 | 10 | .....do | May 24, 1 fruit was growing | 0 | |
| 4 | 9 | 3 p. m., Feb. 25 | .....do | 9 | .....do | .....do | 1 | Seeds germinated well. |
| 5 | 9 | 4 p. m., Feb. 25 | 4 p. m., Feb. 25 | 10 | .....do | May 24, 3 fruits were growing | 3 | Do. |
| 6 | 9 | 10 a. m., Feb. 26 | 10 a. m., Feb. 26 | 10 | Mellitensis navel, St. Michael pollen | May 24, 2 fruits were growing | 0 | Limb bearing fruits died. |
| 7 | 9 | 11 a. m., Feb. 26 | 11 a. m., Feb. 26 | 10 | .....do | May 25, 3 fruits were growing | 3 | An average of 6 seeds per fruit; 4 planted, of which 3 germinated. |
| 8 | 9 | 11.30 a. m., Feb. 26. | 4 p. m., Mar. 6 | 9 | .....do | May 25, 4 fruits were growing | 1 | An average of 5 seeds per fruit; 11 planted, all of which germinated. |
| 9 | 9 | 12.30 p. m., Feb. 26. | .....do | 4 | .....do | May 25, 1 fruit was growing | 1 | Fruit contained 9 seeds; 9 planted, of which 7 germinated. |
| 10 | 5 | 2 p. m., Mar. 2 | 2 p. m., Mar. 2 | 10 | .....do | May 25, 5 fruits were growing | 5 | An average of 4 seeds per fruit; 11 planted, of which 8 germinated. |
| 11 | 5 | 3 p. m., Mar. 2 | 11 a. m., Mar. 7 | 10 | .....do | May 25, 3 fruits were growing | 3 | An average of 11 seeds per fruit; 16 planted, of which 13 germinated. |
| a12 | 5 | 11 a. m., Mar. 9 | 11 a. m., Mar. 9 | 10 | Magnesium sulphate substituted for pollen. | May 25, all 10 found fallen | 0 | |
| a13 | 5 | 12 m., Mar. 9 | Never | | Emasculated and bagged but never pollinated. | .....do | 0 | One became ¼ inch in diameter before falling. |
| a14 | 5 | 2 p. m., Mar. 9 | 2 p. m., Mar. 9 | 10 | Magnesium sulphate substituted for pollen. | .....do | 0 | |
| a15 | 5 | 3 p. m., Mar. 9 | Never | | Emasculated and bagged but never pollinated. | .....do | 0 | |

a Experiments performed on tree of St. Michael, a seedy variety.

### EXPERIMENTS WITH TOMATO BLOSSOMS.

The work so far having been performed on flowers the pistils of which are naturally protected against a premature pollination, it was desired to try some flower the stigma of which is naturally exposed to pollination before it is receptive. It is to be supposed that flowers of the latter kind would not be killed by premature pollination as tobacco flowers are, for nature could not expose the young flowers to so imminent a danger of destruction without the plant becoming extinct or its manner of flowering changed by natural selection. The tomato blossom was found to present the conditions desired, for here the young stigma is exposed more or less for two or three days before it is receptive. (See Pl. IV, fig. 9.)

All of the work on tomato flowers was performed in the greenhouse, where there were grown eight different varieties. These vines grew very vigorously and were pruned and trained to vertical wires as the growth proceeded. Near the conclusion of the work the vines had reached a height of 8 or 9 feet and had such a mass of foliage that pruning was frequently necessary. The following notes of the 25 experiments performed with tomato blossoms are given in about the words written in the notebook as the work progressed. Some explanations and references to figures are inserted, and to avoid too many repetitions the wording has been changed in some places, and the description of the kind of blossom buds chosen is omitted except in experiments 1 and 5. Experiment 5 was performed on fully open flowers, as explained in the notes of that experiment. The kind of blossom buds chosen for all the other experiments is explained in the notes of experiment 1 and illustrated by Pl. IV, fig. 10. Fig. 11 of the same plate shows another of the same age which has been decapitated, and fig. 12 one of same age that has been emasculated. It will be noticed that decapitation prepares the flower for premature pollination just as well as emasculation, but this process of decapitation can not be used in hybridization work since there is danger of the flower becoming self-fertilized by the pollen remaining in the decapitated flower. The flowers were usually simply decapitated because decapitation was more quickly performed and perhaps of less injury to the flowers, although the experiments do not show any injurious effects from emasculation (decapitation employed in experiments 3 and 4, and emasculation in similar experiments 7 and 9). (See Table IV, p. 35.) However, in all experiments where it was necessary to remove all of the pollen the flowers were decapitated and then emasculated. In some cases one experiment is an exact repetition of another, while in other cases it is a repetition so far as practical results are concerned, but different in minor details, as, for example, in the variety of tomato from which the pollen was taken. In all these experiments, except experiment 5, the flowers and resulting fruits were kept covered with paper bags from the commencement of the work until the fruit was ripe.

*Experiment 1.*—March 1, 2 p. m. Ten young flowers of Lorillard variety were decapitated and pollinated at once with pollen from Atlantic Prize. Only young buds with a total length of one-half inch and with their sepal lobes still touching at their apexes were chosen. (Pl. IV, fig. 10, shows the size and appearance of the flower buds chosen for this and all following experiments except experiment 5.) The sepal lobes of these flowers were at this time one-eighth of an inch longer than the petals, stamens, and pistils, which were all of about equal length. In unmolested flowers similar to these it was observed that from this age on the pistils grew more rapidly than the other parts, and usually protruded one-sixteenth to one-eighth of an inch beyond the mature stamens (Pl. IV. fig. 8).

March 19. A few of these flowers were to-day examined, and it was found that some had fallen from the plants, while others were ready to fall at a touch.

April 3. These 10 flowers were all found loose at the base of the stem, and the flowers black, dried, and shriveled.

Results of the experiment: No fruits set.

*Experiment 2.*—March 1, 2.30 p. m. This experiment is in every particular an exact repetition of experiment 1, and the 10 flowers examined March 19 and April 3 showed results exactly the same as those of experiment 1.

*Experiment 3.*—(Compare with experiment 4 and contrast with 1 and 2.) March 1, 3 p. m. These 10 flowers on the same plants as flowers of experiments 1 and 2 were decapitated and then bagged to be pollinated with Atlantic Prize pollen when the pistils have become mature, as shown by other blossoms of the same age to-day, labeled to determine the rapidity of growth of unmolested flowers on these vines.

March 7. On this date the blossoms labeled to indicate the rapidity of growth showed reflex petals and receptive stigmas. An examination of the flowers of experiment 3 showed the decapitated sepals to be reflexed and the stigmas receptive as near as could be determined with a hand lens. These 10 flowers were accordingly pollinated with fresh pollen of Atlantic Prize.

April 3. These 10 flowers were to-day examined and 9 large green tomatoes found developing. One blossom had fallen without setting fruit, but 2 of the 9 large tomatoes were on this same stem, which might possibly be the cause of the falling of the other flower, as 2 tomatoes were enough for so small a stem to support. These fruits now range from 1 to 3 inches in diameter.

Results of the experiment: June 8. Nine large ripe tomatoes.

*Experiment 4.*—(Compare with experiment 3 and contrast with 1 and 2.) March 1, 4 p. m. This experiment is an exact repetition of experiment 3.

March 7. These 10 blossoms treated similarly to those of experiment 3.

April 3. Nine large green tomatoes found; 1 flower had fallen without setting fruit.

Results of the experiment: June 8. Nine large ripe tomatoes.

*Experiment 5.*—March 1. In order to ascertain the percentage of blossoms which would set fruit if pollinated in the manner usually employed in growing tomatoes under glass for commercial purposes, 10 fully opened blossoms were thus pollinated and labeled, but left unbagged. These 10 flowers were of the Lorillard variety and were pollinated in a hasty manner with pollen of Atlantic Prize by simply touching the protruding pistils with a watch glass into which pollen had been shaken from flowers of the latter variety.

April 3. These 10 flowers were examined and 8 large green tomatoes found developing, 1 flower having fallen without setting fruit, and another in the same fruit cluster was still attached and had a strong stem and large green calyx, but the ovary was exceedingly small and had not begun to develop. The last 2 mentioned are in the same fruit cluster with 2 of the 8 large green tomatoes, and this fact may be an explanation of their nondevelopment, since 2 large tomatoes are as many as so small a stem can well support.

Results of the experiment: Eight large well-formed tomatoes.

*Experiment 6.*—(Compare with experiments 1, 2, and 8 and contrast with 7.) March 11, 10 a. m. Ten flowers of the Lorillard variety were decapitated and the stamens entirely removed. The blossoms were then immediately pollinated with fresh pollen of Atlantic Prize.

April 3. Eight of these 10 flowers have fallen loose at the base of the stem, and the flowers are black and shriveled, while the other 2 are still attached by strong fleshy stems. The ovaries, though not enlarged, are plump and green, like tiny tomatoes.

June 10. Two small ripe tomatoes gathered and found to contain 8 seeds each.

Results of the experiment: Two small ripe tomatoes.

This experiment differs from experiments 1 and 2 only in that the flowers were emasculated rather than simply decapitated.

*Experiment 7.*—(Compare with experiments 3 and 4 and contrast with 6 and 8.) March 11, 11 a. m. Ten flowers of the same variety and in every particular similar to those of experiment 6 were decapitated and the stamens entirely removed and the flowers bagged to be pollinated when the pistils become receptive as indicated by other flowers of the same age to-day, labeled to show the rapidity of development.

March 16, 2 p. m. These 10 blossoms were pollinated with fresh pollen of Atlantic Prize.

April 3. The above 10 blossoms examined and 8 good green tomatoes found developing, 1 flower having fallen while the other was still attached by a strong fleshy stem, but the small green ovary was undeveloped.

Results of the experiment: Eight good tomatoes.

*Experiment 8.*—(Compare with experiments 1, 2, and 6 and contrast with 3, 4, 7, and 9.) March 11, 12 m. Ten young blossom buds on Suttons Best of All were decapitated and the stamens entirely removed, and the stigmas covered immediately with fresh pollen of Atlantic Prize.

April 3. These 10 flowers examined and all found fallen loose at the base of the stem and black and shriveled.

Results of the experiment: No fruits.

*Experiment 9.*—(Contrast with experiment 8.) March 11, 12.30 p. m. Ten blossom buds of the same variety, and in all respects similar to those of experiment 8, were similarly treated in regard to emasculation and decapitation, but were bagged without pollination. These 10 flowers are to be pollinated with fresh pollen of Atlantic Prize when the pistils become receptive as indicated by other labeled blossoms of the same age.

March 16, 2.30 p. m. These 10 flowers were pollinated with fresh pollen of Atlantic Prize, the decapitated petal lobes being reflexed, showing that the flowers would have been fully opened had they been left unmolested.

April 3. Ten good large tomatoes found developing.

Results of the experiment: Ten large, well-formed tomatoes.

It will be seen by contrasting experiments 8 and 9 that they differ only in the time of the application of the pollen to the pistils. In experiment 8 the pollen was applied to the immature pistils, while in experiment 9 it was applied to mature pistils.

It being noticed that several of the varieties in the greenhouse were setting a few fruits, although there had been no opportunity for the blossoms to become pollinated, the question naturally arose as to whether tomatoes will set fruit if secluded from wind and insects, and experiments 10 and 11 were undertaken in order to throw some light on this point.

*Experiment 10.*—March 16, 3 p. m. This experiment consisted simply in bagging 10 young blossom buds on the variety Livingston's Beauty. The blossom buds were

unopened and in the same condition as those described in the preceding experiments. In bagging, two blossoms were placed in each bag.

May 9. An examination showed two small fruits, the other flowers having fallen without setting fruits.

Results of the experiment: Two small fruits.

As it was not known at this time that the tomato sometimes sets fruit without the flowers having received pollen, no further attention was given to these small fruits. As shown by later work, however, it is possible that these fruits set without receiving any pollen and, of course, if such was the case they were without seeds. No definite conclusions can be drawn from this experiment, since the two fruits may have set without their flowers having been pollinated at all, or by means of self-fertilization, or by one flower becoming pollinated with pollen from the other flower in the same bag.

*Experiment 11.*—This experiment was performed at the same time and is a repetition of experiment 10, except that it is on the variety Atlantic Prize. The experiment resulted in the falling of nine of the blossoms without setting fruit, while the tenth remained green, with a thick green stem and calyx, but failed to develop a fruit.

Results of the experiment: No fruits.

The results thus far having definitely shown that good results could not be obtained by the immediate pollination of the immature pistils, the question arose as to the cause of the failure—whether the pistils were injured by the premature application of pollen, or whether the pollen had lost its vitality before the pistils became mature. In order to test this point some further experiments were undertaken, beginning with experiment 12. If the pistils were uninjured by the application of pollen at the time the flowers were decapitated, it is evident that another pollination when the pistils became receptive would give as good results as in those experiments in which the flowers received but the one pollination—that at the time when the pistils were receptive.

*Experiment 12.*—(Compare with experiment 14 and contrast with 13.) March 19, 10 a. m. Ten flowers of Sutton's Best of All were decapitated and at once pollinated with fresh pollen of Lorillard. Other similar flower buds were labeled at the same time in order to show the time when the pistils became receptive.

March 27, 9 a. m. These 10 flowers were again pollinated with fresh pollen of Lorillard. The decapitated calyx lobes were reflexed and the pollen of the previous pollination could still be seen upon the stigmas.

April 3. An examination of several of these flowers showed that about half had fallen, while the others seemed to be setting fruit.

May 9. Seven fine fruits were found growing.

Results of the experiment: Seven good fruits.

This experiment indicates that the immature pistils were uninjured by the first pollination, and the failure to obtain fruits in the preceding experiments in which the pistils were prematurely pollinated must be accounted for in some other way, perhaps by the loss of vitality in the pollen.

*Experiment 13.*—(Compare with experiments 3, 4, and 7 and contrast with 6, 12, and 14.) March 19, 10.30 a. m. Ten flower buds on the same variety, and in all respects similar to those of experiment 12, were decapitated and bagged without pollination, to be pollinated with pollen of Lorillard when the stigmas become receptive.

March 27, 9.30 a. m. These 10 flowers were pollinated with fresh pollen of Lorillard. The stigmas appeared receptive when viewed through a hand lens and the pollen adhered readily to them.

April 3. An examination of a few of these flowers showed that some small tomatoes were forming, but their development was not sufficiently advanced to determine results.

May 9. Six good, large fruits were found growing.

Results of the experiment: Six good, large fruits.

*Experiment 14.*—(Compare with experiment 12 and contrast with 13.) March 19, 12 m. This experiment is a repetition of experiment 12, except that the pollen used was from different flowers of the same variety instead of from those of the Lorillard variety.

March 27, 11 a. m. The flowers were again pollinated with fresh pollen from different flowers of the the same variety. One of the 10 was found fallen and another, which was of a yellowish color, about ready to fall.

Results of the experiment: May 9, seven good fruits.

Experiment 15 was planned to be a repetition of experiments 7 and 9 and to contrast with experiment 14, in order to determine whether the flowers are injured by premature pollination. However, when these flowers were receptive and were being pollinated, it was discovered that some of them had become injured in some way after being emasculated, while others could not be found, thus making it necessary to give up experiment 15.

A comparison of experiment 14 with experiments 7 and 9 suggests that early pollination has no detrimental effect upon the pistils, but as experiments 7 and 9 were performed at a different time and pollen from a different variety was used, the conditions are not similar enough to warrant such a conclusion. In order to get more conclusive proof that premature pollination does not injure the young pistils and prevent them from performing their functions when they become mature, experiments 16 and 17 were performed.

*Experiment 16.*—(Compare with experiments 12 and 14 and contrast with 13.) April 3, 2 p. m. Ten young blossom buds of the Lorillard variety were decapitated and immediately pollinated with fresh pollen of Livingston Potato Leaf. When the pistils have become mature, as indicated by other flowers of the same age to-day, labeled to show the rapidity of development, these 10 flowers are to be again pollinated with fresh pollen of Livingston Potato Leaf.

April 11, 9 a. m. The 10 blossoms were again pollinated with fresh pollen of Livingston Potato Leaf. The flowers were fresh with decapitated calyx and corolla lobes reflexed and the pistils apparently receptive, although still bearing the pollen of the first pollination.

May 9. Nine good, large fruits were found developing.

Results of the experiment: Nine good, large fruits.

*Experiment 17.*—(Compare with experiments 7, 9, and 13 and contrast with 12, 14, and 16.) April 3, 3 p. m. Ten young flower buds on the same plants and in all

respects similar to those of experiment 16 were decapitated and bagged without being pollinated, but are to be pollinated with fresh pollen of Livingston Potato Leaf when the pistils become mature.

April 11, 10 a. m. These 10 flowers were pollinated with fresh pollen of Livingston Potato Leaf. The flowers were fresh with decapitated calyx and corolla lobes reflexed and the pistils apparently receptive. No difference in appearance was noticed between these flowers and those of experiment 16.

May 9. Ten good, healthy green fruits found growing.

Results of the experiment: Ten good fruits.

On the same day, and of course before the results of experiments 16 and 17 were known, experiments 18 and 19 were commenced. It was thought likely that experiment 17 would give so much better results than experiment 16 that it would be necessary to explain in what way the first pollination in experiment 16 and similar experiments had injured the pistils. If the injury was due, as in the case of tobacco flowers, to the growth of pollen tubes down the immature pistils, substances other than pollen would not act in that way; while if the injury should prove to be due simply to the coating on the stigmas, some other substance might have the same injurious effect. Since quite a number of ripe tomatoes had been examined and found to contain no seeds, it was thought that this tendency to set fruit without pollination might be increased by the irritation of the stigmas by the application of some substance other than pollen. Consequently in some of the following experiments magnesium sulphate was employed in the place of pollen.

*Experiment 18.*—April 3, 4 p. m. This experiment is similar to experiment 16 in all respects except that magnesium sulphate was used in place of fresh pollen to coat over the surface of the stigmas immediately after decapitation. Ten flower buds of the same variety and in all respects similar to those of experiment 17 were decapitated and the pistils dipped at once into powdered magnesium sulphate, and the flowers bagged.

April 11, 10.30 a. m. The 10 flowers were pollinated with fresh pollen of Livingston Potato Leaf. The darkened appearance of the stigmas of the flowers when pollinated indicated some injury due to the magnesium sulphate.

May 9. Three good fruits found developing. Two of the blossoms were still fresh and green, with large stems and large green calyx lobes, but had failed to set fruit, while the rest of the 10 flowers had fallen without setting fruit.

Results of the experiment: Three good fruits.

*Experiment 19.*—April 3, 4.30 p. m. Ten blossom buds of the same variety and in all respects similar to those of experiment 18 were decapitated, and the pistils dipped at once into powdered magnesium sulphate and the flowers bagged.

April 11. The 10 flowers were examined and found in the same condition as those of experiment 18 at this time.

May 9. Two of the blossoms were found still fresh and green with large stems and large, green calyx lobes, but they had failed to set fruit, while the other 8 blossoms had fallen without setting fruit.

Results of the experiment: No fruits.

*Experiment 20.*—April 3, 5 p. m. Five blossom buds of Lorillard and 5 of Sutton's Best of All were emasculated and bagged.

April 11, 11.30 a. m. The stigmas of these blossoms were coated with magnesium

sulphate. The 10 blossoms were in good, fresh condition, with reflexed calyx and corolla lobes and protruding pistils showing fresh receptive stigmas.

May 9. One small green tomato found developing. Three of the flowers were found with large green stems and calyxes, but failed to set fruit. The other 6 were found fallen without having set fruit.

Results of the experiment: One ripe seedless tomato 1¼ inches in diameter.

The results of this experiment gave additional proof that the seedless tomatoes previously found growing in the greenhouse had resulted from flowers that had never been pollinated. Experiment 22 was an exact repetition of experiment 20, but resulted in the falling of all the flowers without setting fruit.

It having been shown by experiments 12, 14, and 16 that the failure to obtain fruits in experiments 1, 2, and 8 was not caused by the presence of the pollen on the immature pistils, it would seem to be due to loss of vitality of the pollen before the pistils became receptive. To test this point experiments 21 and 22 were performed.

*Experiment 21.*—(Contrast with experiment 17.) May 9, 4 p. m. Ten blossom buds of Sutton's Best of All were emasculated and bagged to be pollinated when the pistils become mature with pollen of Lorillard collected to-day at 5.30 p. m.

May 14, 1.30 p. m. These 10 flowers were to-day pollinated with pollen of Lorillard collected May 9 and placed in a watch-glass closely covered and kept in the greenhouse near the tomato vines in order that it might be exposed to the same conditions of temperature, etc., as the pollen placed upon the immature pistils in the previous experiments. The decapitated sepals and petals of these flowers were reflexed and the pistils had a good healthy appearance when pollinated.

June 6. Eight of the flowers were found detached below the ovaries, which had not enlarged after pollination. One flower set a fruit which attained a size of 2⅛ inches in diameter and contained 22 seeds. The other flower could not be found.

Results of the experiment: One fruit.

The fact that experiments 6 and 21 produced 3 small fruits containing but few seeds shows that the pollen had not lost quite all its vitality during the five days from the time it was collected till the pistils on which it was placed became mature.

*Experiment 23.*—(Contrast with experiment 16.) May 9. Ten blossoms of Sutton's Best of All were emasculated and at once pollinated with fresh pollen of Lorillard. Some of the pollen was reserved for a second application to these flowers when the pistils become mature.

May 14, 2 p. m. The 10 flowers were pollinated with pollen of Lorillard collected May 9 and kept in the greenhouse since that time. When pollinated the second time all parts of the flowers were in a fresh, healthy condition, the stigmas showing white from the application of pollen May 9.

June 9. All of the flowers were found detached below their ovaries, which had not enlarged since pollination.

Results of the experiment: No fruits.

*Experiment 24.*—(Compare with experiment 25 and contrast with 20 and 22.) May 14, 3 p. m. Ten young blossom buds of Sutton's Best of All were emasculated and bagged. These blossoms are never to be pollinated.

June 6. All of the 10 blossoms were found fallen below the ovaries, which had not enlarged.

Results of the experiment: No fruits.

*Experiment 25.*—This experiment is an exact repetition of experiment 24, except that it was performed on the Lorillard variety.

Results of the experiment: No fruits.

While the work with tomato blossoms indicates no injurious effects from the growth of pollen tubes down the immature pistils, it proves the fallacy of the commonly accepted opinion that when pollen is placed on young stigmas it will remain there and fertilize the flower when the pistils finally become receptive. It is also shown that tomato blossoms will sometimes, though seldom, set fruit without pollination, and the number of fine, large, seedless, and almost seedless tomatoes that grew on the vines under glass without any attention having been paid to the pollination of the flowers shows that the absence or scarcity of pollen on the stigmas will sometimes result in the production of better, firmer, and less seedy tomatoes than those which result from a natural pollination. An examination of tomato flowers growing out of doors showed that the stigmas of the fully opened flowers were completely covered with pollen, and tomatoes on the same vines consequently were found to contain the usual large number of seeds. One of these of average size—3 inches in diameter and 2½ inches thick—and not unusually seedy, contained 308 seeds; while some of the same variety and of the same size that were grown under glass contain no seeds, and others but a dozen or two. The fine tomatoes above mentioned, some of which contained no seeds and the others but few, grew on large thrifty vines which bore but a few fruits each, owing to the failure of their flowers to receive pollen. Had these vines borne at the time a crop of tomatoes containing the normal number of seeds it is likely that the ones without seeds, as well as those with but few seeds, would not have reached the size they did. As it was, however, the tomatoes without seeds reached a diameter of 2½ and 3 inches and were firm and of excellent quality. If some of the fruits on a vine have set as a result of a liberal pollination, while others have set as a result of a slight pollination, or even without pollination, it is natural to suppose that the nourishment of the vine would go largely to the fruits containing an abundance of seeds, while seedless ones would be dwarfed. That such is the case has been shown by the experiments of Munson[a] and Bailey[b]. The same effect is shown by Pl. IV, fig. 16, which is a photograph of a cluster of Wilmot Hamburg grapes grown under glass. The small berries are ripe and have the same color and flavor as the large ones, but are entirely seedless and doubtless set without pollination. The large berries contain several seeds each and likely resulted from blossoms that were naturally pollinated, perhaps by insects that found their way into the hothouse.

When there is competition on the same plant between seedless and seeded fruits the latter receive the most nourishment and become the

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[a] Ann. Rep. Maine Exp. Station, 1892, p. 50.

[b] Rep. Cornell Univ. Exp. Station, 1891, p. 53.

larger, but by careful breeding it is not unlikely that a tomato can be obtained that will produce seedless fruits as large as the seeded fruits we now have. The large size of seedless bananas and pineapples indicates that this is possible. But since the most convenient way of propagating the tomato at present is by means of seeds, it is not a tomato entirely seedless that is wanted, but a large, well-shaped, firm tomato with but few seeds.

While a comparison of the experiments in which the flowers were emasculated and never pollinated with the experiments in which the flowers were emasculated and magnesium sulphate substituted for pollen would seem to suggest that the irritation of the receptive stigmas may stimulate the setting of fruits, yet fruits set so rarely without flowers having received pollen that a very large number of flowers would have to be tested in order to determine whether the mere irritation of the stigmas by the application of substances other than pollen has any tendency to cause fruits to set.

TABLE IV.—*Experiments with tomato blossoms.*

[Each experiment performed upon 10 similar flower buds.]

| Experiment number. | Pistils would have been receptive in— | When emasculated or decapitated. | When pollinated. | Remarks regarding pollen and pollination. | When examined, with remarks on condition. | Number of ripe fruits. | Remarks. |
|---|---|---|---|---|---|---|---|
| | *Days.* | 1901. | | | | | |
| 1 | 6 | 2 p. m., Mar. 1 | 2 p. m., Mar. 1 | Fresh Atlantic prize pollen | Mar. 19, some fallen, others falling | 0 | Apr. 3, all fallen and dry. |
| 2 | 6 | 2.30 p. m., Mar. 1 | 2.30 p. m., Mar. 1 | do | do | 0 | Do. |
| 3 | 6 | 3 p. m., Mar. 1 | Mar. 7 | do | Mar. 19, some green tomatoes noticed. | 9 | June 8, 9 large ripe tomatoes. |
| 4 | 6 | 4 p. m., Mar. 1 | do | do | Apr. 3, 9 large green tomatoes | 9 | June 8, ripe and gathered. |
| 5 | Receptive. | Never | Mar. 1 | do | Apr. 3, 8 large green tomatoes | 8 | Pollinated rapidly and not bagged. |
| 6 | 5 | 10 a. m., Mar. 11 | 10 a. m., Mar. 11 | do | Apr. 3, 8 found fallen, 2 very small | 2 | June 10, fruits very small, 8 seeds each. |
| 7 | 5 | 11 a. m., Mar. 11 | 2 p. m., Mar. 16 | do | Apr. 3, 8 green tomatoes | 8 | |
| 8 | 5 | 12 m., Mar. 11 | 12 m., Mar. 11 | do | Apr. 3, all found fallen | 0 | |
| 9 | 5 | 12.30 p. m., Mar. 11. | 2.30 p. m., Mar. 16. | do | Apr. 3, 10 good tomatoes growing | 10 | |
| 10 | 5 | Never | Never | Bagged March 16 to test self-fertilization | May 9, 2 small fruits | 2 | Two flowers to each bag. |
| 11 | 5 | do | do | do | May 9, 9 found fallen | 0 | One large green calyx. |
| 12 | 8 | 10 a. m., Mar. 19 | 10 a. m., Mar. 19; 9 a. m., Mar. 27. | Fresh Lorillard pollen each time | May 9, 7 good fruits growing | 7 | |
| 13 | 8 | 10.30 a. m., Mar. 19. | 9.30 a. m., Mar. 27. | Fresh Lorillard pollen | May 9, 6 good fruits growing | 6 | |
| 14 | 8 | 12 m., Mar. 19 | 12 m., Mar. 19; 11 a. m., Mar. 27. | Fresh Sutton's Best of All pollen each time | May 9, 7 good fruits growing | 7 | |
| 15 | 8 | 12.30 p. m., Mar. 19. | | Some lost and some had become injured, and so could not be pollinated. | | | |
| 16 | 8 | 2 p. m., Apr. 3 | 2 p. m., Apr. 3; 9 a. m., Apr. 11. | Fresh Potato Leaf pollen each time | May 9, 9 good large fruits growing | 9 | |
| 17 | 8 | 3 p. m., Apr. 3 | 10 a. m., Apr. 11 | Fresh Potato Leaf pollen | May 9, 10 good large fruits growing | 10 | |
| 18 | 8 | 4 p. m., Apr. 3 | 4 p. m., Apr. 3; 10.30 a. m., Apr. 11. | Powdered magnesium sulphate used Apr. 3, and fresh Potato Leaf pollen Apr. 11. | May 9, 3 good green fruits growing | 3 | Magnesium sulphate injured young pistils. |
| 19 | 8 | 4.30 p. m., Apr. 3 | 4.30 p. m., Apr. 3 | Powdered magnesium sulphate | May 9, 8 fallen, 2 with green calyxes. | 0 | |
| 20 | 8 | 5 p. m., Apr. 3 | 11.30 a. m., Apr. 11. | do | May 9, 1 fruit, 6 fallen, and 3 with green calyxes. | 1 | Fruit ripened and was seedless. |
| 21 | 5 | 4 p. m., May 9 | 1.30 p. m., May 14. | Lorillard pollen 5 days old | June 6, 1 fruit growing | 1 | Small, contained 22 seeds. |
| 22 | 5 | 5 p. m., May 9 | 12 m., May 14 | Magnesium sulphate substituted for pollen | June 6, all fallen | 0 | |
| 23 | 5 | May 9 | May 9, 2 p. m.; May 14. | Fresh Lorillard pollen used May 9, and Lorillard pollen 5 days old May 14. | June 6, all found fallen | 0 | |
| 24 | 5 | 3 p. m., May 14 | Never | Emasculated and bagged; never pollinated | do | 0 | |
| 25 | 5 | 4 p. m., May 14 | do | do | do | 0 | |

## CONCLUSION.

The following summary of results obtained with tobacco, cotton, orange, and tomato flowers contrasts the effects of immature and mature pollinations. The orange work does not lend itself well to this comparison, for it is unsafe to say that the young pistils were not receptive when pollinated:

| Prematurely pollinated. | Resulting number of fruits. | Maturely pollinated. | Resulting number of fruits. |
|---|---|---|---|
| 80 tobacco blossoms | 3 | 40 tobacco blossoms | 38 |
| 60 cotton blossoms | 32 | 40 cotton blossoms | 33 |
| 60 orange blossoms, 6 experiments | 15 | 42 orange blossoms, 5 experiments | 9 |
| 40 tomato blossoms | 2 | 70 tomato blossoms | 60 |

To determine whether cotton bolls would set without pollination, or by the substitution of other substances for pollen was not attempted, but a summary of this work with the other three flowers is as follows:

| Emasculated and stigmas covered with substances other than pollen. | Resulting number of fruits. | Emasculated and bagged without pollination. | Resulting number of fruits. |
|---|---|---|---|
| 60 tobacco blossoms | 14 | 20 tobacco blossoms | 2 |
| 20 orange blossoms, seedy variety | 0 | 20 orange blossoms, seedy variety | 0 |
| 30 tomato blossoms | 1 | 20 tomato blossoms | 0 |

Much has been said in apology for the lack of success of attempted hand pollinations, advocating perseverance, and regarding it as a great accomplishment if even 5 or 10 per cent of the flowers set seed. But this is erroneous, for if one is working with plants that naturally cross readily and is not trying to make a cross between two very different plants, the results should certainly not be considered successful unless a greater percentage of fruits is obtained than set on the same plants naturally without artificial pollination. We increase the production of fruits by giving attention to plants in other respects, and likewise by a careful study of the flowers upon which we work we can obtain greater percentages of fruits by hand pollination, including emasculation, than set naturally. After the behavior of tobacco, cotton, and tomato flowers was quite well understood, and they were correctly operated upon for obtaining the best results, the following percentages of fruit were obtained:

| Flowers hand pollinated. | Fruits obtained. | Per cent. |
|---|---|---|
| 40 tobacco (experiments 7, 16, 20, and 36) | 38 | 95 |
| 40 cotton (experiments 2, 4, 7, and 10) | 33 | 82 |
| 60 tomato (experiments 3, 4, 7, 9, 13, and 17) | 52 | 86 |

The writer is fully convinced that persons having hand-pollinations to make with unfamiliar flowers, or with flowers with which their previous work has been unsatisfactory, will find it profitable to make some small experiments in order to determine the method to which the flowers respond with a large percentage of fruits. These experiments can usually be made with the proper pollen and flowers so that the fruits will contain seed of the desired kind.

While attention is constantly being called to the many adaptations of flowers for accomplishing various purposes, such as cross-fertilization, it might be well to attempt to discover if some adaptations for preventing premature pollination have not been brought about by natural selection. That many flowers are admirably formed to successfully prevent premature pollination is certain, but that such devices have been brought about for this purpose will perhaps never be certain. Proterogenous flowers that open or unfurl in blooming usually


Fig. 1.—Flowers of *Sabbatia angularis:* (*a*) Appearance while pollen is being shed; (*b*) Stigma and anthers avoiding each other; (*c*) Receptive stigma and deciduous anthers. (From drawings by E. E. Lower.)

do so when their pistils have reached a receptive condition. The pistils of such flowers are perfectly protected from pollen from all sources until they are receptive. Pl. IV, fig. 6, shows a fully open, receptive begonia flower, while fig. 7 shows the two calyx lobes securely closed over the young pistil of a similar but younger flower. Proterandrous flowers open while the pistils are immature, but even here modifications are met with which insure the pistils against premature pollination. The stigmatic surfaces are often folded together, thus preventing pollination even though the immature pistil be exposed. Fig. 1 shows the interesting behavior of flowers of *Sabbatia angularis; a* shows the condition of the flower when the anthers discharge their pollen, two petals and one stamen having been cut away in order to show the young pistil, which at this stage has no stigmatic surface exposed. Instead of growing straight out and thus coming in contact with the drying anthers, the pistil invariably grows out to one side, passing between the filaments of the stamens. The stamens at this stage begin to bend to the side of the flower opposite the one occupied

by the pistil, as shown by *b*, fig. 1, and by the time the pistil straightens and exposes its stigmatic surfaces the stamens have assumed a recumbent position and have drooped their anthers, as shown by *c*. The modifications of this proterandrous flower successfully prevent self-fertilization and premature pollination without interfering with the proper shedding of pollen.

The many adaptations of flowers to insure pollination at a certain time is interesting and suggestive of the importance to be attached to the time for applying pollen when hand-pollinating. Movements on the part of stamens so as to bring the pollen to the pistil at a certain stage in the growth of the flower, and especially movements on the parts of the pistils themselves which bring the stigmas into contact with the stamens, are admirable adaptations. With some flowers, such as those of corn, the pistils have no definite length, but continue to grow in search of pollen, as it were. If pollinated upon protruding from the husks, corn silks perform their functions, turn brown, and become dry without making a growth of more than 2 or 3 inches beyond the end of the ear, but if kept secluded from pollen they will continue to grow for a week and will attain a length greater by 14 or more inches than would otherwise have been the case.

During the work with the various flowers herein mentioned nothing has been more noticeable than the individuality possessed by flowers of different forms. It seems impossible to say that the flowers of one plant will respond in a given manner because those of another plant so respond. From these experiments it is evident that flowers of different genera of the same order, as the tomato and tobacco, respond differently to the effects of premature pollination, while the flowers of *Datura tatula*, of the same order, suffer as do those of tobacco, but do not fall.

Two of the five kinds of flowers experimented with mature their pistils before their stamens and were uninjured by premature pollination, while the three that mature their anthers and stigmas at the same time were injured. It is perhaps accidental that such is the case with these five flowers, but it is to be hoped that future work will reveal some means of distinguishing flowers that will, from those that will not produce seed when prematurely pollinated. For the plant breeder it is quite important to know with what flowers one can apply the pollen at the time of emasculation and thus avoid the extra labor of removing the bags, pollinating, and again bagging the flowers when the pistils have become receptive.

It is apparent that several of the phenomena suggested by the results of these experiments have not been sufficiently proven. However, concerning the following points the writer feels no doubt:

That the application of good tobacco pollen to immature tobacco pistils causes the flowers so treated to fall from the plant because of the growth of pollen tubes into their ovaries;

That tobacco and tomato plants sometimes set and ripen fruits without the flowers having received any pollen, and that such fruits contain no germinative seeds:

That but few fruits will be obtained by the pollination of immature cotton and tomato pistils, but that good percentages may be obtained if the pollination is performed when the pistils are receptive.

The lesson taught by these experiments is that some flowers can not be successfully pollinated when the work of emasculation is performed, while others can, and that no arbitrary mode of procedure can be given for all flowers. A study of the behavior of each kind of flower will reveal its peculiarities and its requirements, and, understanding these, hand-pollinations should be highly successful.

# PLATES.

## PLATE I.

*Figs. 1 and 2.*—The upper portions of the same two main stems of a growing tobacco plant.

The photograph for fig. 1 was taken 3 p. m., June 21, 1900, immediately after the numbered flowers had been emasculated and pollinated, and the lettered flowers had been emasculated but not pollinated.

The photograph for fig. 2 was taken 3 p. m., June 23, or just forty-eight hours later than that for fig. 1, and shows that premature pollination has caused flowers 1, 2, and 6 to fall, while the more mature flowers, 3, 4, and 5, show no injury from pollination, but, on the contrary, were fecundated and set seed pods, as shown in Pl. II, fig. 1.

Flowers 1 and 2, fig. 1, would not have been receptive for two days and flower 6 for three days, and according to results of experiments they were expected to fall about thirty-six hours after pollination.

Flowers 3, 4, and 5 would have been fully open and receptive in one day and were expected to give a fair percentage of seed pods.

If early emasculation had caused the falling, flowers *c* and *d* should have fallen also.

The advancement of an unmolested flower may be studied by observing the small bud on the right in fig. 1 and its growth as shown by fig. 2 and the forming seed pod in Pl. II, fig. 1.

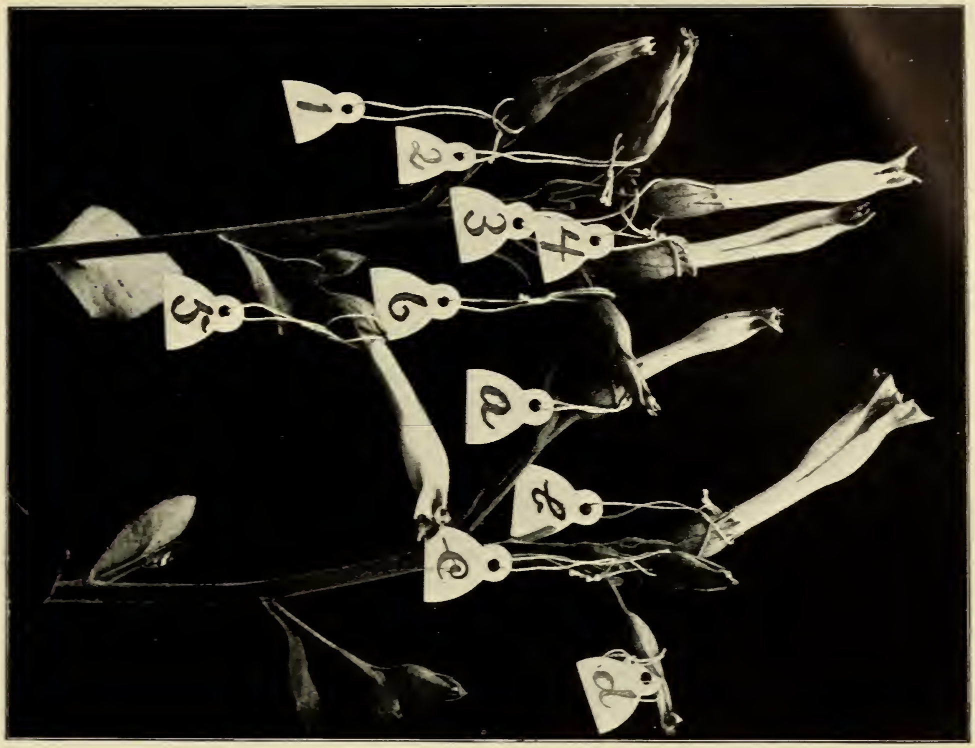


FIG. 1.—GROWING TOBACCO BLOSSOMS AT TIME OF EMASCULATION (THREE-FOURTHS NATURAL SIZE)

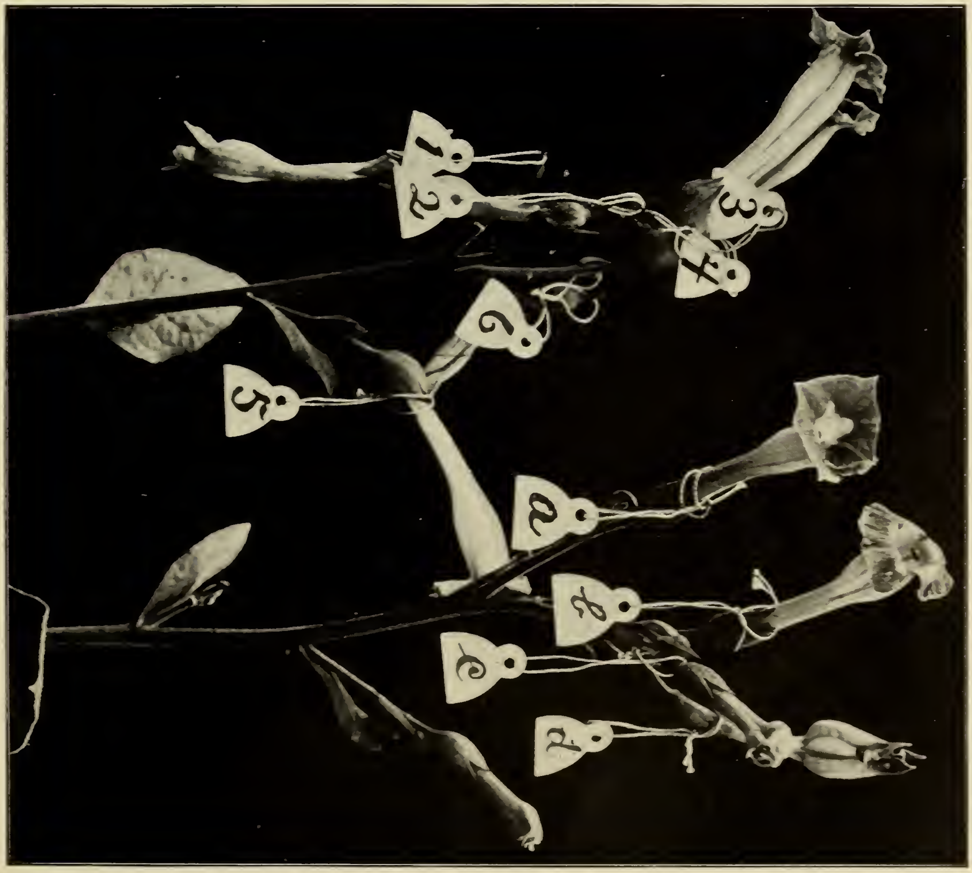


FIG. 2.—SAME BLOSSOMS AS IN FIG. 1, TWO DAYS LATER (THREE-FOURTHS NATURAL SIZE).

## PLATE II.

*Fig. 1.*—The same portion of the same plant shown in Pl. I, photographed eight days later than fig. 1. The emasculated flowers *b* and *c* have not yet fallen, while the seed pods resulting from flowers 3 and 4 have attained considerable size.

*Figs. 2, 3, 4, and 5.*—Photomicrographs of pollen grains and pollen tubes in sections of flowers that fell because of premature pollination.

*Fig. 2.*—Section of a tobacco stigma showing pollen grains which have germinated and sent their tubes into the style. Flower from which this section was made was emasculated and pollinated May 25, 11 a. m., two days before it would have opened. May 27, 11 a. m., it was found fallen and was fixed for microscopic study. ($\times$ 250 diameters.)

*Fig. 3.*—Pollen tubes farther down the style. Figs. 2 and 3 are sections of the same pistils. ($\times$ 315 diameters.)

*Fig. 4.*—Mass of pollen tubes near the point where they enter the ovary from the style. The flower from which this section was made was one of experiment 21 (see Table I). ($\times$ 160 diameters.)

*Fig. 5.*—Pollen tubes in ovary on the placenta at attachment of ovules. Figs. 4 and 5 are from different sections of the same ovary. ($\times$ 75 diameters.)

FIG. 1.—SAME BLOSSOMS AS IN PL. I, FIG. 1, EIGHT DAYS LATER (THREE-FOURTHS NATURAL SIZE).

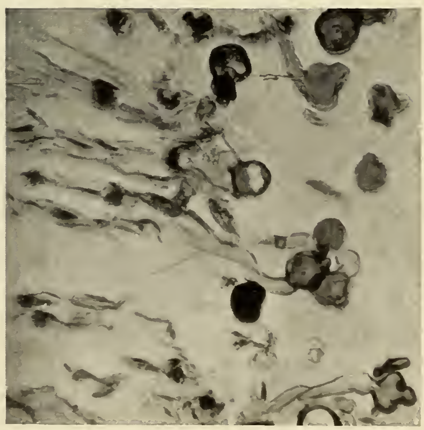


Fig. 2. (× 250.)

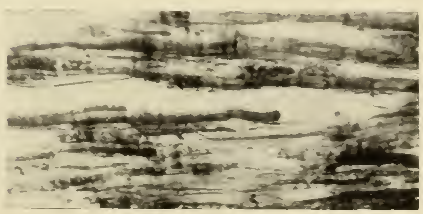


Fig. 3. (× 315.)

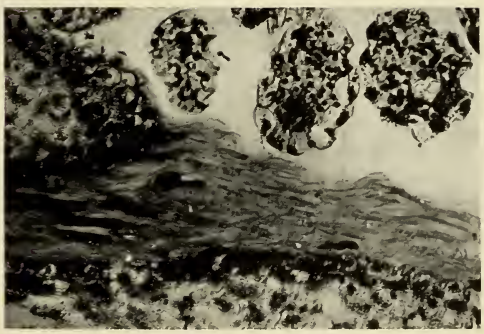


Fig. 4. (× 160.)

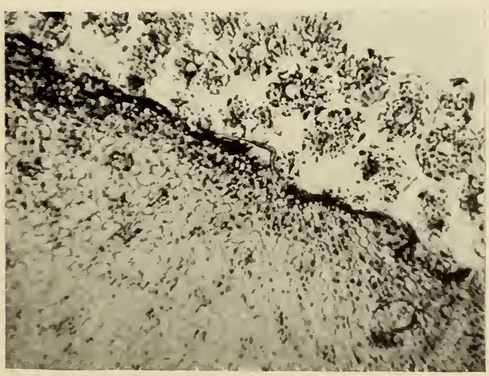


Fig. 5. (× 75.)

PHOTOMICROGRAPHS OF TOBACCO POLLEN TUBES.

## PLATE III.

Flowers 1 and 2 were pollinated March 3, two days before they would have been fully open, No. 2 being emasculated. Thirty-nine hours after being pollinated these flowers had fallen. No. 1 shows the anthers just opening.

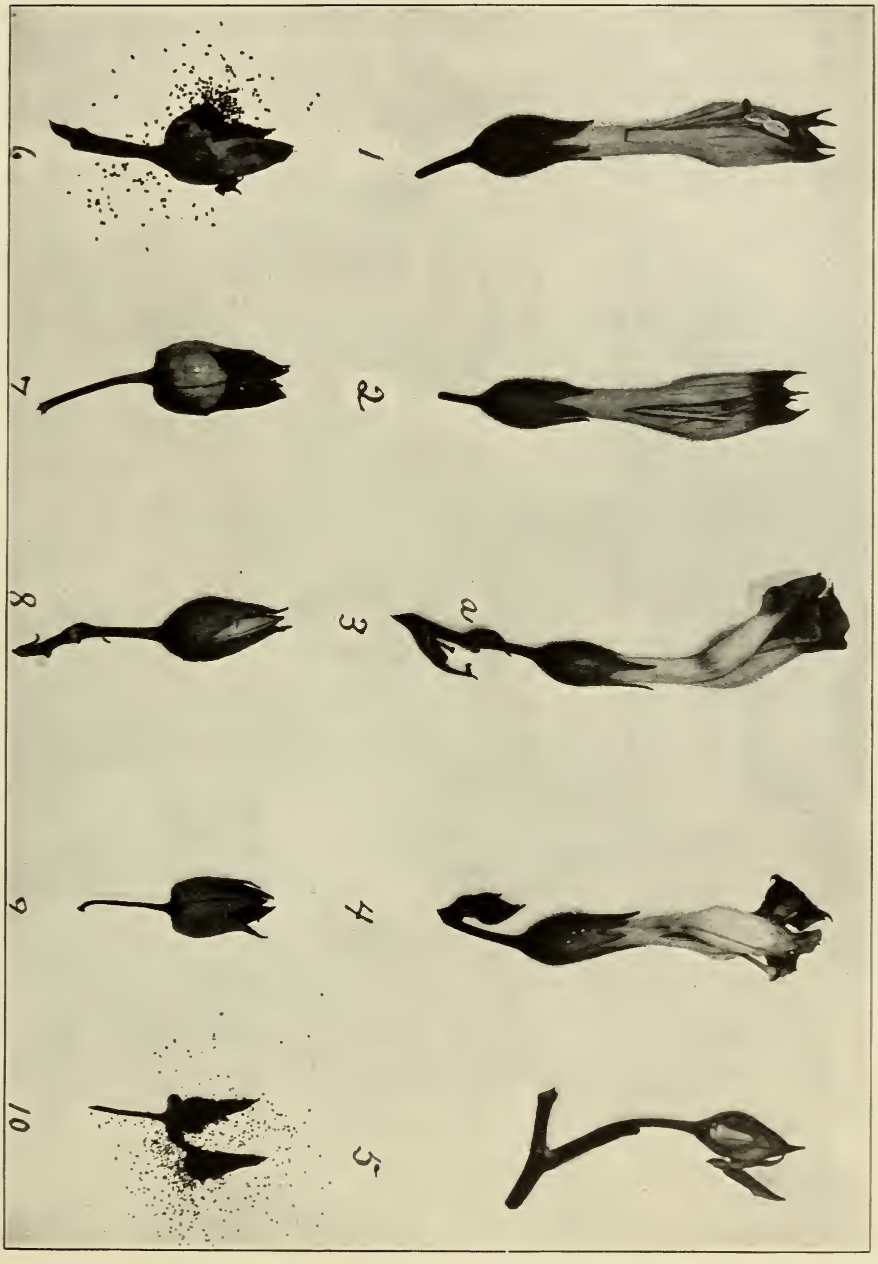
Flowers 3, 4, and 5 were emasculated February 27. No. 3 was not pollinated, while Nos. 4 and 5 had their stigmas coated over with corn flour. March 5, Nos. 3 and 4 were still fresh, while the corolla of No. 5 had fallen and the ovary begun to enlarge.

*No. 6.*—A ripe pod torn open to show normal seeds for comparison with chaffy seeds that sometimes form in pods the flowers of which were not pollinated or had other substances substituted for pollen as in Nos. 9 and 10.

*No. 7.*—A normal ripe seed pod.

*No. 8.*—A green pod formed from a flower which was emasculated and had its stigma coated with air-slacked lime.

*Nos. 9 and 10.*—Ripe pods of experiment 45 in which the stigmas were coated with corn flour. After being photographed pod 9 was examined and found to contain only light, chaffy seed like those shown in the opened pod 10.



TOBACCO BLOSSOMS AND SEED PODS (NATURAL SIZE).

PLATE IV.

*Fig. 1.*—An orange forty-three days after pollen was applied to the stigma.

*Figs. 2, 3, 4, and 5.*—Flowers of a Melitensis navel orange, showing different stages in their development.

*Fig. 2.*—Fully open flower, showing abortive anthers characteristic of seedless varieties. Two petals were removed for convenience in photographing.

*Fig. 3.*—Flower five days younger than No. 2, and the kind used in experiments 10 to 15, inclusive. The stigma at this age bears a large drop of stigmatic fluid.

*Fig. 4.*—Flower nine days younger than No. 2, and the kind used in experiments 1 to 9, inclusive.

*Fig. 5.*—Flower same age as No. 4, showing appearance after emasculation. The stigmas at this early age show stigmatic fluid.

*Fig. 6.*—A fully opened pistillate begonia flower, showing receptive pistil.

*Fig. 7.*—A pistillate begonia flower slightly younger than No. 6 and showing the calyx lobes tightly closed, perhaps to protect the young pistil from premature pollination.

*Fig. 8.*—A fully opened tomato blossom of the Lorillard variety, showing receptive stigma extending a short distance beyond the surrounding stamens, which are about to open and shed their pollen.

*Fig. 9.*—A blossom of Lorillard three days younger than No. 8, showing the stigma already exposed.

*Fig. 10.*—A blossom of Lorillard that would have been fully open like No. 8 in about six days. This shows the kind of buds selected in all the tomato experiments to determine the effects of premature pollination.

*Fig. 11.*—A bud similar to No. 10, which has been decapitated to permit of a premature pollination of the pistil.

*Fig. 12.*—A blossom bud similar to No. 10, which has had its stamens entirely removed.

*Figs. 13, 14, and 15.*—Cotton flowers, showing method of emasculation. The flowers were gathered one day before they would normally have opened. Flower 14 has had the upper portion of corolla cut away ready for emasculation. Flower 15 shows emasculation completed.

*Fig. 16.*—A cluster of Wilmot Hamburg grapes, showing difference in size between normal and seedless berries.



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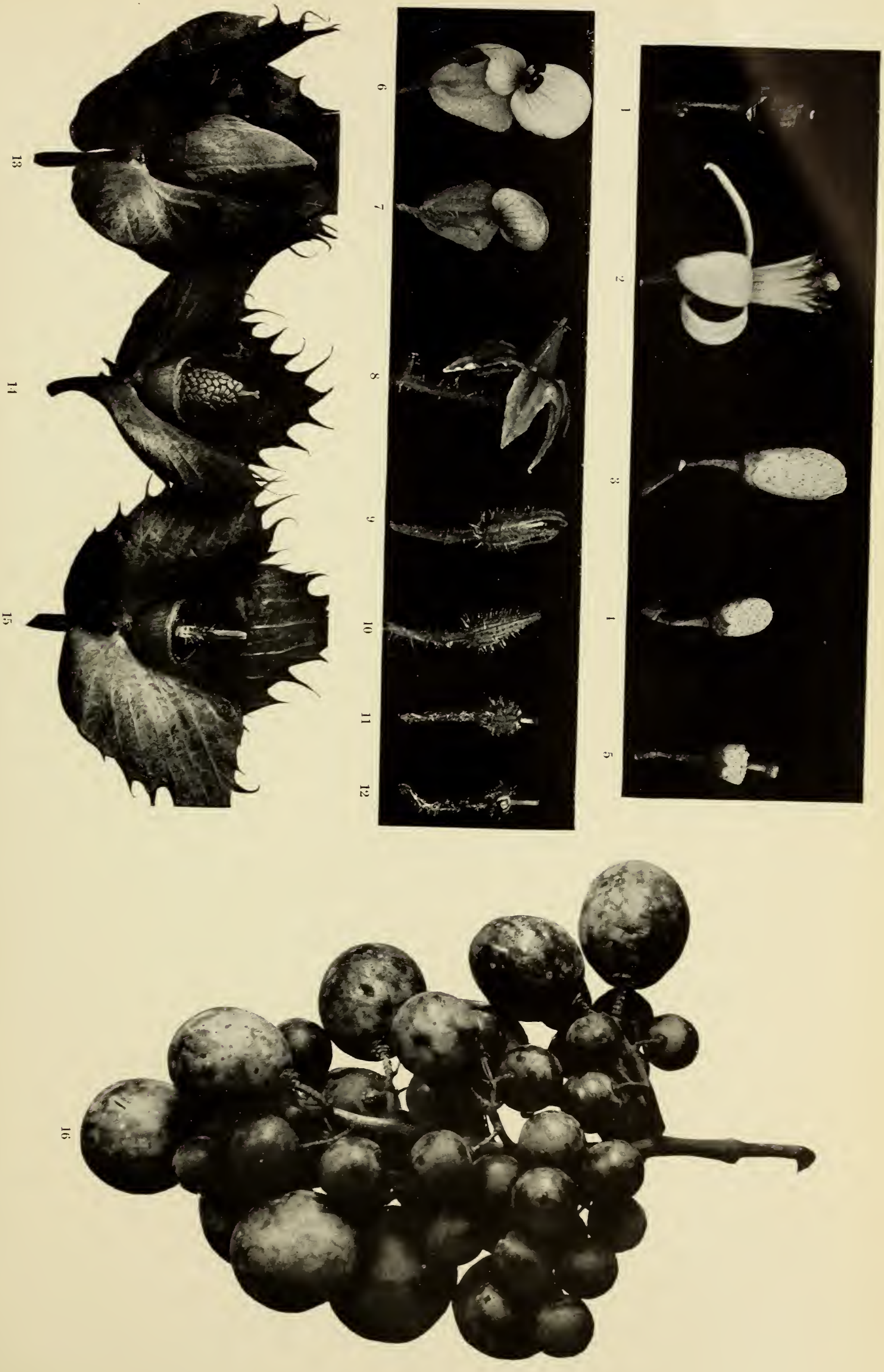


METHODS OF EMASCULATION. ALSO A GRAPE CLUSTER, SHOWING DIFFERENCE IN SIZE BETWEEN SEEDLESS AND SEEDED BERRIES.

## BULLETINS OF THE BUREAU OF PLANT INDUSTRY.

The Bureau of Plant Industry, which was organized July 1, 1901, includes Vegetable Pathological and Physiological Investigations, Botanical Investigations and Experiments, Grass and Forage Plant Investigations, Pomological Investigations, and Gardens and Grounds, all of which were formerly separate Divisions, and also Seed and Plant Introduction, The Arlington Experimental Farm, Tea Investigations and Experiments, and the Congressional Seed Distribution.

Beginning with the date of organization of the Bureau, the independent series of bulletins of the Division of Vegetable Pathology and Physiology, the last number of which was 29, and of each of the other Divisions were discontinued, and all are now published as one series of the Bureau.

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